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ORIGINAL ARTICLE

In utero anti-HBsAg IgG Antibody Transmission from Mother to Foetus is Associated with Protection against Hepatitis B Infection in New-born Babies in Bénin Republic

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ABSTRACT

Background: Hepatitis B is an infectious disease caused by hepatitis B virus (HBV). The virus targets hepatocytes and induce inflammation. Poor management of hepatitis B infection can lead to chronic hepatitis, cirrhosis or even, hepatocellular carcinoma. Unfortunately, HBV can be transmitted from mother to child during pregnancy. In addition, when mothers are immunized, they can passively transfer their immunity to their foetus during pregnancy. The levels of anti-hepatitis B surface antigen (HBsAg) IgG antibody transferred by mothers to their new-born babies before any vaccination were therefore, determined in this study.

Materials and Methods: Sixty six (66) pregnant women from a cohort at Cotonou (n = 54) and at Porto-Novo (n = 12) (Bénin Republic) were enrolled into this study. After obtaining written informed consent, 5 mL of venous blood samples were collected from the mothers and their new-born babies (n = 67) at delivery before any vaccination. Thereafter, IgG levels against Hepatitis B surface antigen (HBsAg) were measured in the sera of the study participants using Enzyme-Linked Fluorescence Assay.

Results: Over 60% of the mothers and 55.22% of the new-born babies have more than 10 mIU/mL of anti-HBsAg specific IgG antibodies. Seven new-born babies were not immunized whereas their mothers were immunized. None of the mothers and the new-born babies was hepatitis B positive.

Conclusion: Mother-to-child passive acquisition of anti-HBsAg specific IgG antibodies could be associated with protection of new-born babies against hepatitis B infection. Evaluation of anti-HBsAg specific IgG antibodies in new-born babies is therefore suggested to guide vaccination.

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INTRODUCTION

Hepatitis B virus (HBV) infection is an important public health challenge. The World Health Organization (WHO) estimated that 296 million people were living with chronic hepatitis B in 2019 while 1.5 million people worldwide are estimated to be infected with HBV each year (1,2). WHO estimation showed that in 2019, 820, 000 people have died from hepatitis infection complications, including liver diseases such as hepatic cirrhosis and hepatocellular carcinoma (HCC) (3,4).

The prevalence of HBV infection is highly different throughout the world with high prevalence areas defined as region with more than 8% of the population tested positive for HBsAg (5). As the prevalence in Benin is under 2%, Benin is considered as a low prevalence area (6).

Perinatal transmission is one of the modes of HBV transmission from mother to foetus and to new-born (1). During pregnancy, mainly maternal IgG are transferred to foetus through the placenta. IgG1, with certain specificities and with high avidity, are more preferentially and efficiently transferred to the foetus than others (7). This transfer begins around 18 weeks of gestation and increases during the following weeks, reaching 100 – 120% of mother's level at birth (8,9). The *in utero* transfer of IgG occurs through active transportation across the placental barrier to the foetus via the Brambell receptor commonly called FcɣRn (10). These IgG antibodies are involved in new-born protection and mediate antimicrobial and antibody-related cytotoxicity (11). They recognize microbes on intestinal mucosa but also in the circulation and tissues (12,13)

Placental IgG transfer confer an important immunity on the new-born babies during the first months of life (8,14,15). The passive immunization of the foetus which is particularly pronounced during the 3rd trimester reflects the mother's immunological experience. This antenatal protection is optimized by many parameters including term delivery, adequate maternal specific antibody levels, antigen immunogenicity and healthy placenta (8). The infant's protection is further reinforced by breastfeeding (16). Passive immunity from mother to foetus and new-born is of extreme importance for adaptation of the neonate to the extra-uterine environment.

Levels of anti-HBsAg IgG antibody between 10 - 100 IU/mL are considered to be protective and the protective role of anti-HBs is correlated with 79% lower odds of reactivation (17). Indeed, the natural course of HBV infection is determined by the interplay between viral replication and host immune parameters. Antibodies against HBsAg are involved in neutralization of HBV by clearing up circulating HBsAg and infectious HBV particles from the peripheral blood (18).

Based on the aforementioned factors, better knowledge of placental anti-HBsAg IgG transfer will allow accurate HBV prevention in new-born babies. Therefore, this study was designed to determine at delivery, the levels of anti-HBs antibodies transferred by mothers to their new-born babies before vaccination.

MATERIALS AND METHODS

Study area and cohort

Pregnant women (n = 66) were recruited into the study from two health facilities in Southern Benin (Bethesda and the Deo Gratias Hospitals). These women gave birth to 67 babies and all the babies were enrolled into the study. The study was conducted from May 2019 to December 2019.

Sample and clinical data collection

At delivery, 5 mL of venous blood was obtained from each mother and new-born baby, dispensed into plain sample tubes and serum obtained was stored at -20°C. Samples were obtained from the new-born babies within 24 hours of delivery and before any vaccination. In addition to the blood sample, demographic and clinical data such as age, term of pregnancy, HBV vaccination status, medical, surgical and obstetrical history were collected using a standard questionnaire.

Exclusion criteria

The following were excluded from the study: vaccinated new-born babies, preterm babies and their mothers, mothers or new-born babies with life-threatening complications, mothers (and their babies) with Human Immunodeficiency Virus (HIV) or tuberculosis infection.

Ethical consideration

This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and

was approved by the Institutional Review Board of the Department of Biochemistry and Cellular Biology of University of Abomey-Calavi (No 69/11-08-2015). Also, written informed consent was obtained from all the pregnant women that participated in this study.

Quantification of anti-HBsAg IgG antibody by Enzyme Linked Fluorescence Assay (ELFA)

Specific IgG antibody against hepatitis B surface antigen (HBsAg) were quantified using VIDAS® Anti-HBs Total II (#30318, Biomerieux). The reaction steps are performed automatically by the mini VIDAS automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) principles. Briefly, after the dilution of the sera, 200 µL are aspirated and discharged inside the coated tips with HBsAg. The anti-HBs antibodies present in the sample will bind to the HBs antigens fixed inside the cone. Washing steps remove the unbound compounds. In the second step, mouse alkaline phosphatase-conjugated monoclonal anti-human IgG is drawn into the cone and binds to human IgG bound to HBs antigens. In the final development step, 4-methyl-ombelliferyl phosphate is drawn into the coated tips and pushed back out. The alkaline phosphatase catalyses the hydrolysis of the 4-methyl-ombelliferyl phosphate to 4-methyl-ombelliferone. This reaction product emits a fluorescence measured at 450 nm. Finally, the mini VIDAS generates antibody concentrations expressed in mIU/mL. Anti-HBsAg IgG concentration greater than or equal to 10 mIU/mL is considered positive in accordance with the international reference rules of the Centre for Disease Control and Prevention (19).

Statistical analyses

Data were collected and analysed using Microsoft Excel 2010 and GraphPad (San Diego, CA, USA).

RESULTS

We included 66 mothers and 67 new-born babies as there was a set of twins among the infants. Unfortunately, we were only able to obtain clinical and biological data from 35 mothers- and their new-born babies (n = 35, average birth weight = 2994 ± 251 g). Only one mother was vaccinated against hepatitis B before her pregnancy and had more than 10 mIU/mL of anti-HBsAg IgG antibody. Neither the mother nor the new-born baby was hepatitis B positive. Among these mothers, there were 13 primigravidae (37.14%, mean age 20.84 ± 3.70 years) and 22 multigravidae (62.86% mean age 29.66 ± 5.39 years).

Distribution of mothers according to hepatitis B immunization

Many countries adopted 10 mIU/mL as the lowest titre to indicate protective immunity against HBV infection. However, 100 mIU/mL is considered for long-term protection (20,21). As shown in Figure 1A, 42 out of the 66 mothers (63.64%) had more than 10 mIU/mL anti-HBsAg IgG antibody. Thirty (71.42%) out of the 66 mothers had more than 50 mIU/mL. Among these 30 mothers, 17 (56.66%) had more than 100 mIU/mL while 11 (64.70%) mothers had more than 200 mIU/mL. Of the 11 mothers, four mothers had more than 500 mUI/mL anti-HBsAg IgG with two of them having more than 1000 mIU/mL anti-HbSAg IgG antibody.

Considering the babies, 37 out of the 67 new-born babies (55.22%) had more than 10 mIU/mL anti-HBsAg IgG antibody. Of these 37 babies, 26 (70.27%) had more than 10 mIU/mL with 9 (34.61%) having more than 200 mIU/mL. Of the 9 babies with over 200 mIU/mL anti-HBsAg IgG antibody, 6 (66.66%) had more than 500 mIU/mL anti-HBsAg IgG antibody (Figure 1B).

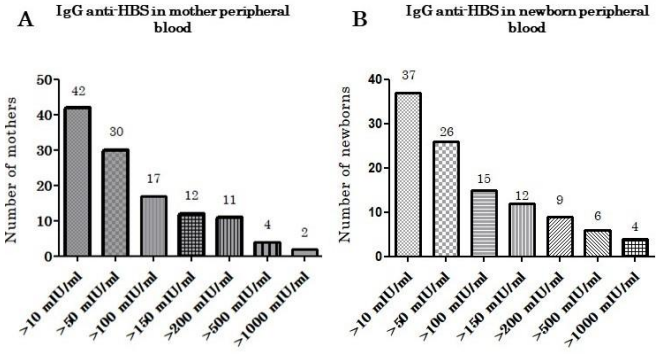

Figure 1: Distribution of mothers (A) and new-born babies (B) according to anti-HBs IgG antibody levels.

Distribution of new-born babies according to their mother's immune status

We related the concentration of each mother to that of their respective new-born. Figure 2 shows that 94.59% of the immunized new-born babies had their mothers immunized while 5.41% of the immunized new-born babies had their mothers

non-immunized. On the other hand, 7 (23.33%) of the non-immunized new-born babies (n = 7) had their mothers immunized and 76.67% of the non-immunized new-born babies had their mothers non-immunized (Table 1).

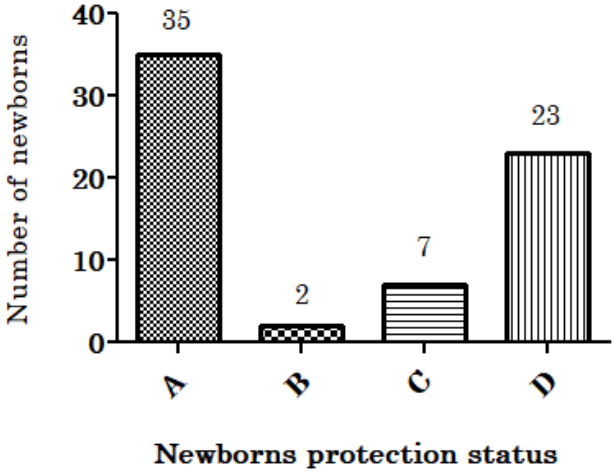

Figure 2: Distribution of new-born babies according to their protection status
A: Number of immunized new-born babies from immunized mothers,
B: Number of immunized new-born babies from non-immunized mothers, C: Number of non-immunized new-born babies from immunized mother,
D: Number of non-immunized new-born babies from non-immunized mothers

Table 1: Anti-HBsAg IgG levels in immunized mothers against HBV and in their non-immunized children

Anti-HBsAg IgG antibody (mIU/ml)							
Mothers	97*	12	14	27	50		101
Babies	3 8	3	7	7	3		3

*Mother of twins

DISCUSSION

Our study shows that 63.64% of the mothers immunized against HBV had transferred this immunity to their respective new-born babies. This confirms the *in utero* transfer of antibodies from mother to new-born as reported (8). This transfer occurs essentially after the 28th week of gestation and depends on the gestational age of the mother (8). Furthermore, 23.33% of immunized mothers did not transfer protective immunity against HBV to their new-born babies. Indeed, among mothers who did not transfer protection to their new-born babies, one had a concentration of anti-HBsAg IgG antibody that was more than 100 mIU/mL. Another interesting observation was that a set of twins born of a mother whose anti-HBsAg IgG antibody concentration was 97 mIU/mL had respectively, 3 mIU/mL and 8 mIU/mL of anti-HBsAg IgG antibody concentration. This observation could be the consequence of low levels of FcγRn receptors on syncytiotrophoblast surface. It could also result from the destruction of free IgG in the placenta by lysosomes in a cytoplasmic vesicle (8). The saturation of FcγRn receptors may also inhibit the *in utero* transfer of antibodies. It has been reported that at a certain high concentration of IgG in the mother, the amount transferred to the foetus decreases due to receptor saturation (22,23). This ultimately leads to reduction in the amount of IgG transferred to the foetus. (22,23). Moreover, it has been shown that from an IgG concentration higher than 15 g/L, the transfer of IgG to the foetus is strongly reduced (24). Furthermore, it has also been reported that in Africa, the higher the maternal total IgG concentration, the lower the IgG concentration transferred to the child, and even the less protective it is (25,26). This observation has been related to the environment as studies involving African women living in Europe showed that these women have different characteristics (97,98). In these conditions, vaccinating women during pregnancy without knowing their HBV serology could lead to a very high level of antibodies in some mothers and could result in a decrease in transfer of anti-HBsAg IgG antibody to the new-born babies (24).

Among the mothers who did not have protective immunity against HBV, we observed that 8.33% transferred protective immunity against HBV to their new-born babies. This could be due to the fact that the antibody concentration received by the foetus increased significantly and resulted in a significant decrease of this antibody in the mother. This observation could be explained by the fact that mothers transfer a lot of antibodies to the foetus until they lose protection (22,23).

Our study showed that 97.14% of the immunized mothers who transferred protective immunity against HBV to their new-born babies had not been vaccinated. This observation confirms the natural immunization of the latter. This transfer of

immunity from the mother to the new-born is so important that 70.27% (n = 26) of the immunized new-born babies had anti-HBs IgG antibody level five times higher than the protection threshold. Reports have shown that IgG antibodies transmitted by unvaccinated mothers to their new-born babies are longer-lived than those transmitted by vaccinated mothers to their new-born babies (27–29). Observations from this study, therefore, suggest that the transfer of passive immunity by a naturally immunized mother is more effective and long-lasting than immunity transmitted after vaccination. However, the quantity and quality of maternal IgG transmitted to new-born is influenced by several factors including maternal nutrition and environment (30).

Our study demonstrated the possibility of protection of new-born babies against HBV through prior immunization of their mothers. Further studies with a larger cohort are needed to prove the efficacy of protection of new-born babies against HBV through prior immunization of their mothers. It would be interesting to always check the immune status of women during pregnancy. It would also be interesting to measure the inhibitory power of these antibodies. In addition, there is increasing evidence of vaccine accidents and adverse effects associated with certain vaccine adjuvants such as aluminium salt (31,32). For example, aluminium salt is known to cause macrophage myofasciitis (33). Some of these accidents could be avoided by measuring antibodies against HBsAg before vaccination. Furthermore, cases of deaths of children following the administration of trivalent diphtheria-tetanus-pertussis (DTP) vaccine in Guinea-Bissau was documented (34). Indeed, in a cohort of 2944 children, including 1694 boys and 1250 girls, who received a dose of DTP vaccine, 13% and 16% of deaths were recorded in boys and girls, respectively, after vaccination (34).

The *in utero* transferred antibodies in combination with human breast milk-derived antibodies may reduce the infant's response to vaccines by neutralizing vaccine antigens (reviewed here (12). It is therefore important to monitor the anti-HBsAg antibody levels in new-born babies to determine the appropriate time of vaccination.

CONCLUSION

Our study showed that majority of mothers immunized against hepatitis B had their new-born babies immunized at birth. The natural protection of new-born babies against hepatitis B virus due to previous immunization of their mothers should be taken into consideration in the different vaccination strategies. This passive immunization that protects the new-born until the immune system is fully functional could be reinforced by exclusive breastfeeding, which should be encouraged.

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DECLARATIONS

Conflict of Interest

No competing interest to declare.

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